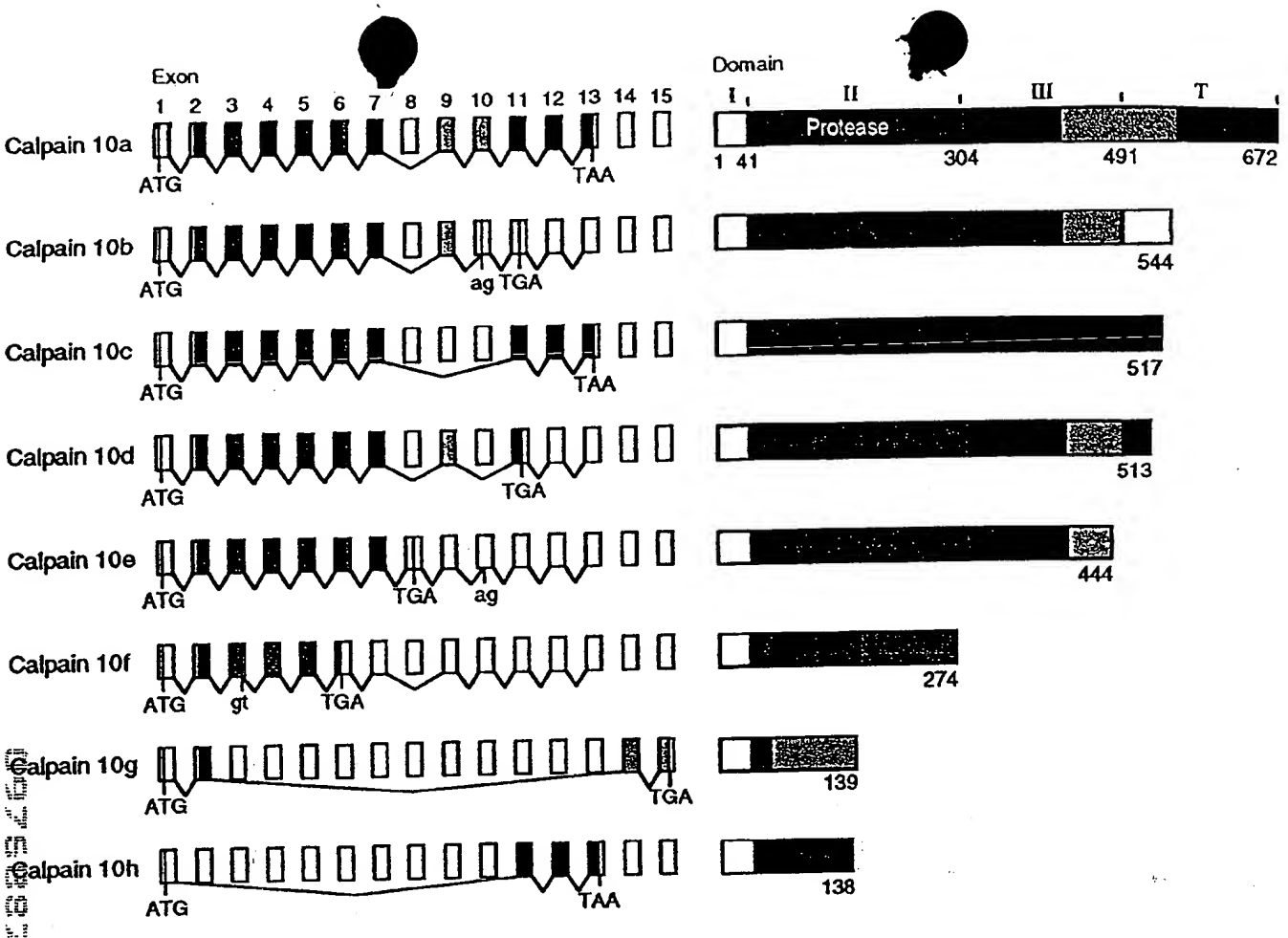

Exon
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Calpain 10a
ATG
TAA
Calpain 10b
ATG
ag TGA
544
Calpain 10c
ATG
TAA
517
Calpain 10d
ATG
TGA
513
Calpain 10e
ATG
TGA
ag
444
Calpain 10f
ATG
gt
TGA
274
Calpain 10g
ATG
TGA
139
Calpain 10h
ATG
TAA
138
Domain
I
II
III
T
Protease
1 41
304
491
672

FIG. 1

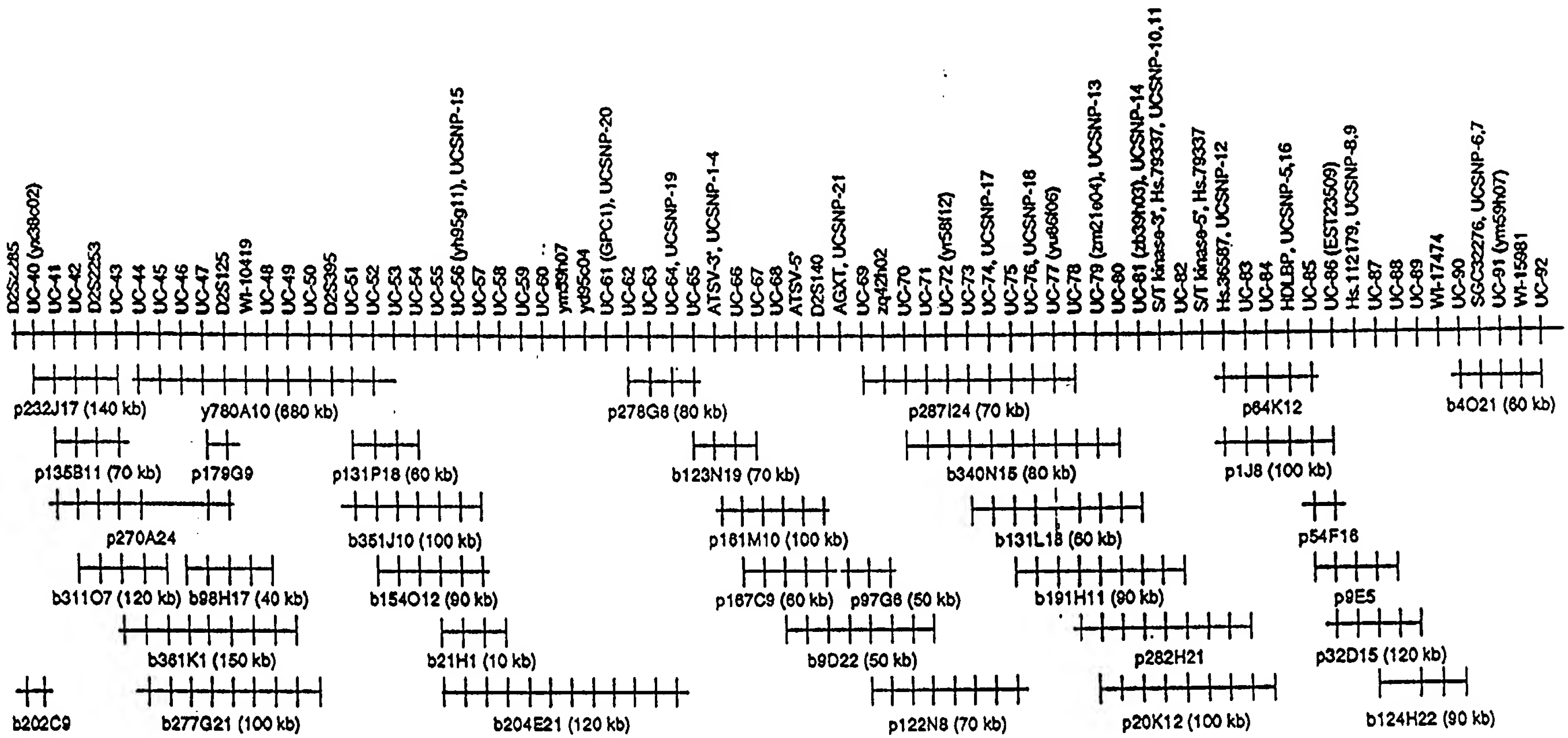

D2S285
UC-40 (yx38c02)
UC-41
UC-42
D2S2253
UC-43
UC-44
UC-45
UC-46
UC-47
D2S125
WI-10419
UC-48
UC-49
UC-50
D2S395
UC-51
UC-52
UC-53
UC-54
UC-55
UC-56 (yh95g11), UCSNP-15
UC-57
UC-58
UC-59
UC-60
ym59h07
yd95c04
UC-61 (GPC1), UCSNP-20
UC-62
UC-63
UC-64, UCSNP-19
UC-65
ATSV-3', UCSNP-1-4
UC-66
UC-67
UC-68
ATSV-5'
D2S140
AGXT, UCSNP-21
UC-69
zq42h02
UC-70
UC-71
UC-72 (yr58f12)
UC-73
UC-74, UCSNP-17
UC-75
UC-76, UCSNP-18
UC-77 (yu86f06)
UC-78
UC-79 (zm21e04), UCSNP-13
UC-80
UC-81 (zb39h03), UCSNP-14
S/T kinase-3', Hs.79337, UCSNP-10,11
UC-82
S/T kinase-5', Hs.79337
Hs.36587, UCSNP-12
UC-83
UC-84
HDLBP, UCSNP-5,16
UC-85
UC-86 (EST23509)
Hs.112179, UCSNP-8,9
UC-87
UC-88
UC-89
WI-17474
UC-90
SGC32276, UCSNP-6,7
UC-91 (ym59h07)
WI-15981
UC-92
p232J17 (140 kb)
y780A10 (680 kb)
p278G8 (80 kb)
p287I24 (70 kb)
p84K12
b4O21 (60 kb)
p135B11 (70 kb)
p179G9
p131P18 (60 kb)
b123N19 (70 kb)
b340N15 (80 kb)
p1J8 (100 kb)
p270A24
b351J10 (100 kb)
p161M10 (100 kb)
b131L18 (60 kb)
p54F18
b311O7 (120 kb)
b98H17 (40 kb)
b154O12 (90 kb)
p187C9 (60 kb)
p97G8 (50 kb)
b191H11 (90 kb)
p9E5
b361K1 (150 kb)
b21H1 (10 kb)
b9D22 (50 kb)
p282H21
p32D15 (120 kb)
b202C9
b277G21 (100 kb)
b204E21 (120 kb)
p122N8 (70 kb)
p20K12 (100 kb)
b124H22 (90 kb)

FIG. 2

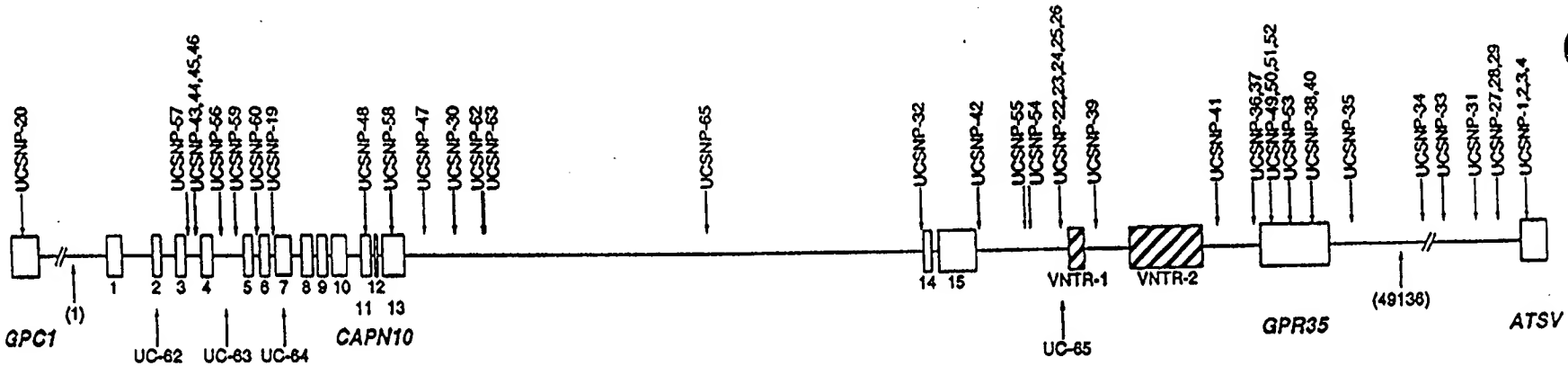


FIG. 3

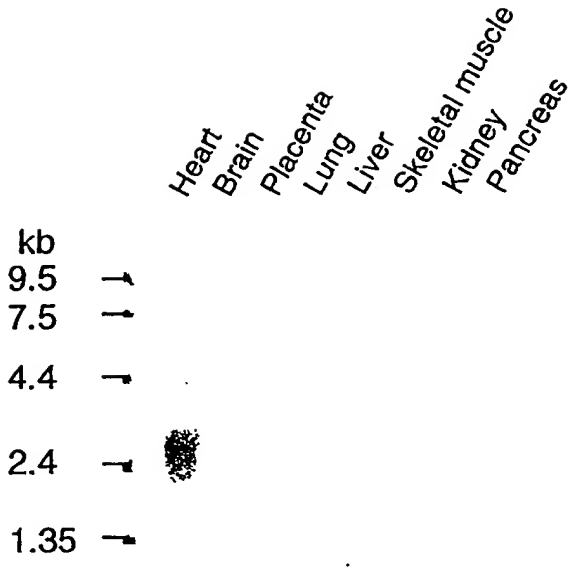

Heart
Brain
Placenta
Lung
Liver
Skeletal muscle
Kidney
Pancreas
kb
9.5
7.5
4.4
2.4
1.35

FIG. 4

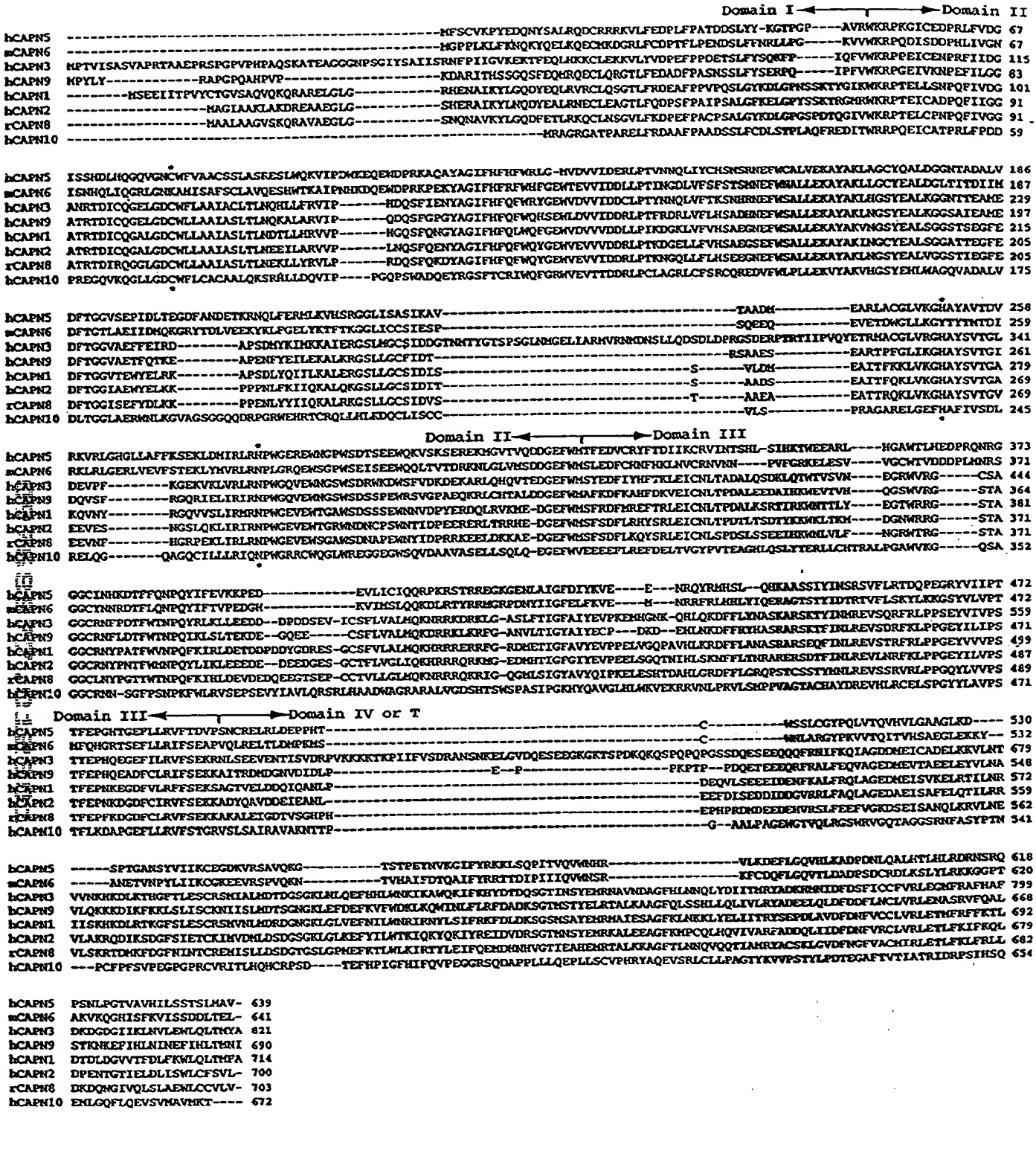

Domain I
Domain II
Domain III
Domain IV or T
hCAPN5
mCAPN6
hCAPN3
hCAPN9
hCAPN1
hCAPN2
rCAPN8
hCAPN10

FIG. 5

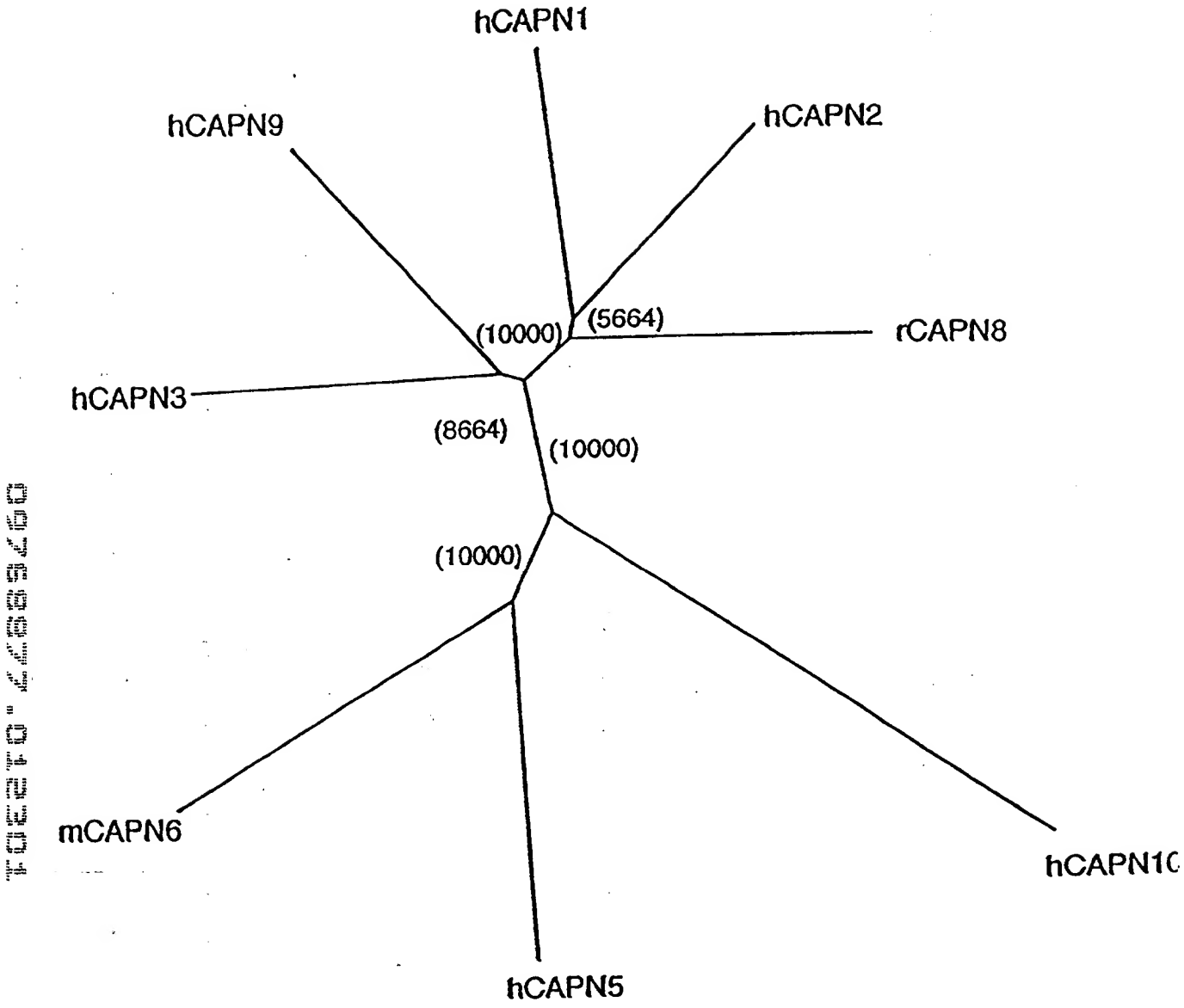

hCAPN1
hCAPN9
hCAPN2
(10000)
(5664)
rCAPN8
hCAPN3
(8664)
(10000)
(10000)
mCAPN6
hCAPN10
hCAPN5

FIG. 6

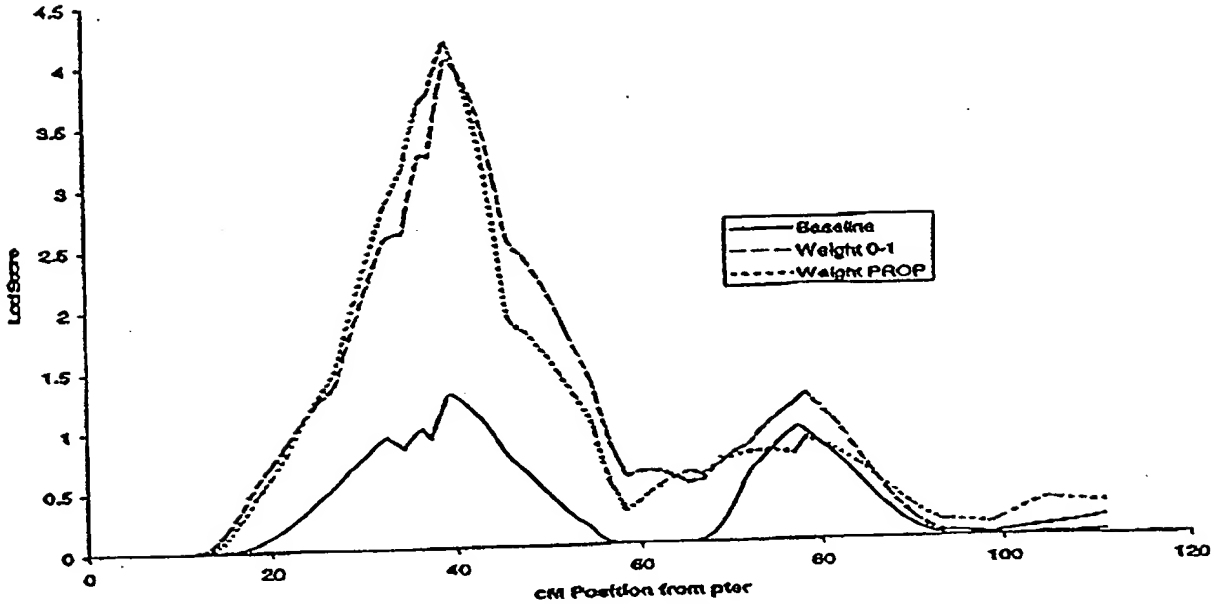

LodScore
cM Position from pter
Baseline
Weight 0-1
Weight PROP

FIG. 7A

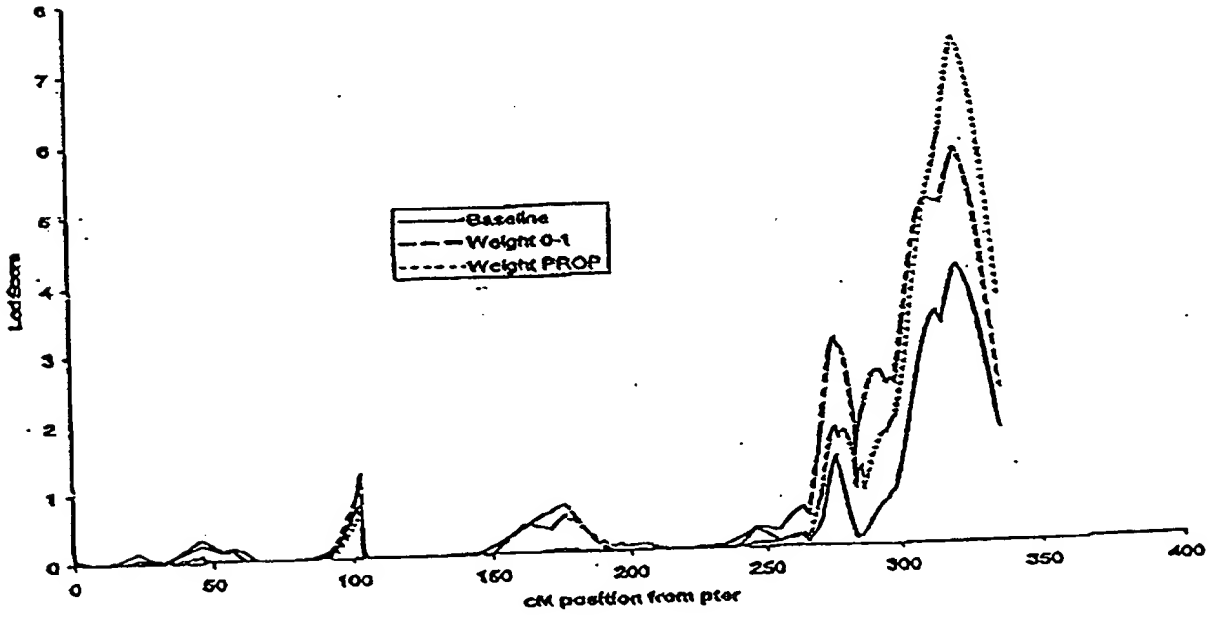

LodScore
cM position from pter
Baseline
Weight 0-1
Weight PROP

FIG. 7B

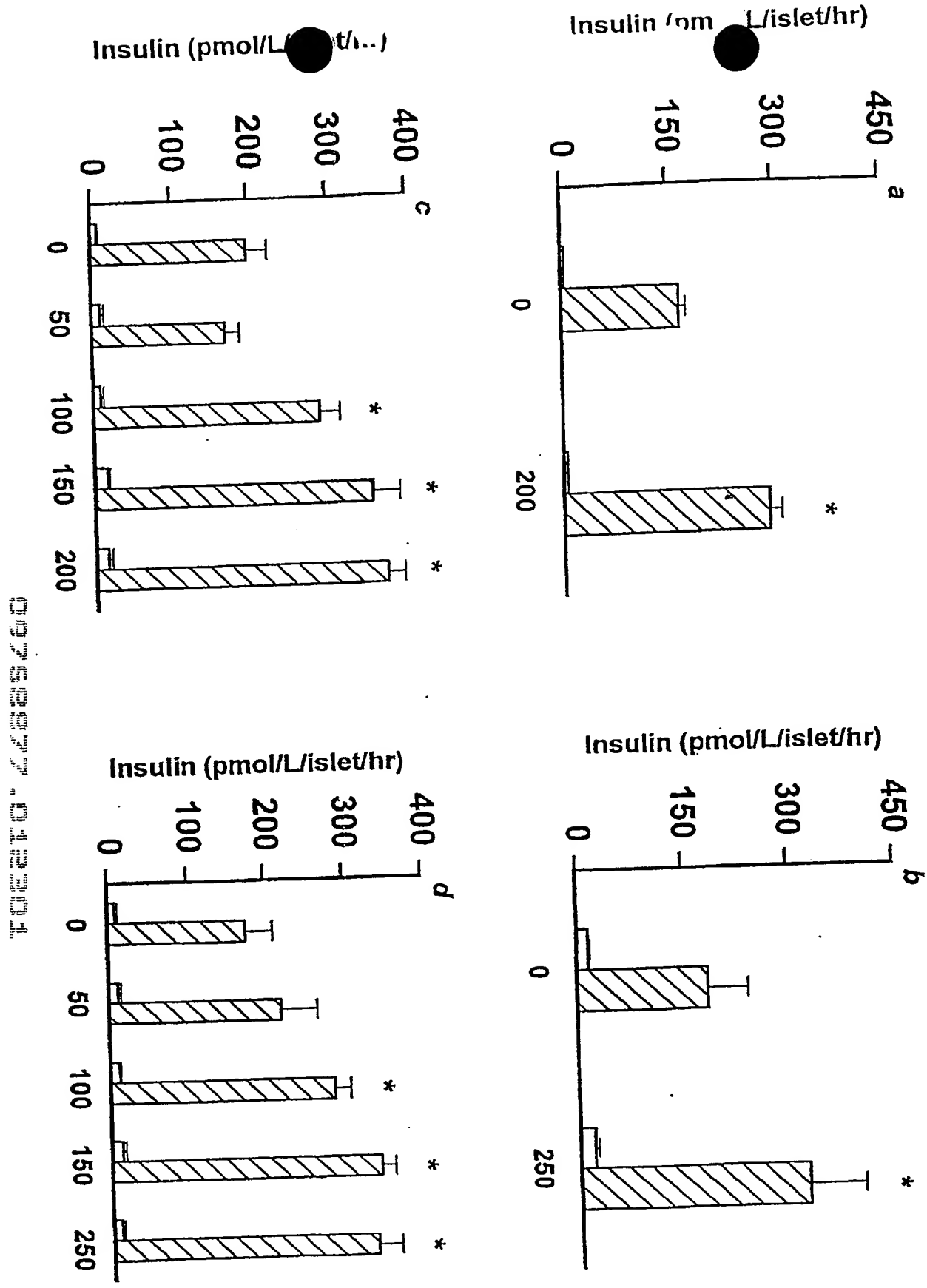

Insulin (pmol/L/islet/hr)
0
150
300
450
a
0
200
*
Insulin (pmol/L/islet/hr)
0
100
200
300
400
c
0
50
100
150
200
*
*
*
Insulin (pmol/L/islet/hr)
0
150
300
450
b
0
250
*
Insulin (pmol/L/islet/hr)
0
100
200
300
400
d
0
50
100
150
250
*
*
*

FIG. 8

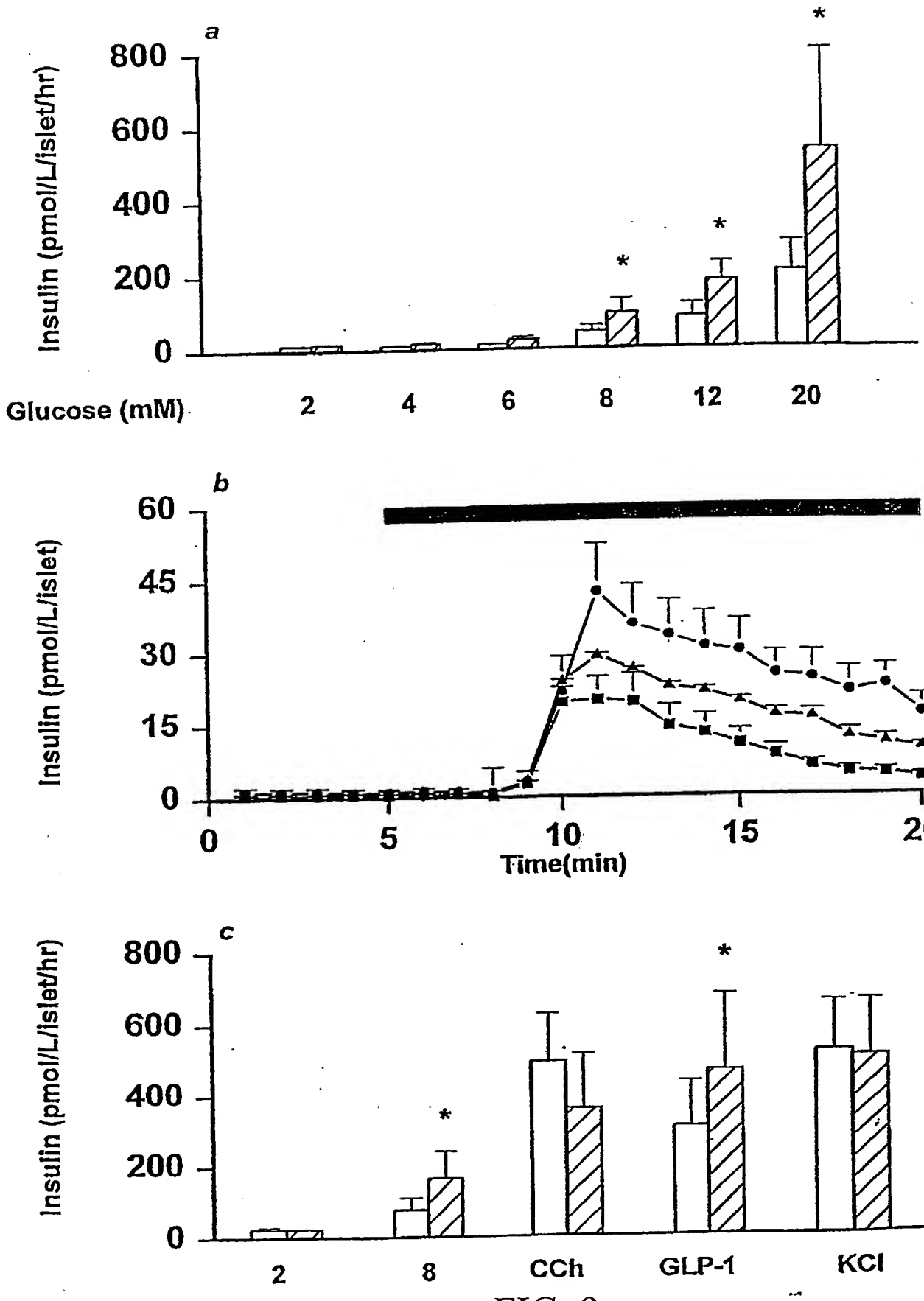

a
Insulin (pmol/L/islet/hr)
800
600
400
200
0
Glucose (mM)
2
4
6
8
12
20
*
b
Insulin (pmol/L/islet)
60
45
30
15
0
0
5
10
15
20
Time(min)
c
Insulin (pmol/L/islet/hr)
800
600
400
200
0
2
8
CCh
GLP-1
KCl

FIG. 9

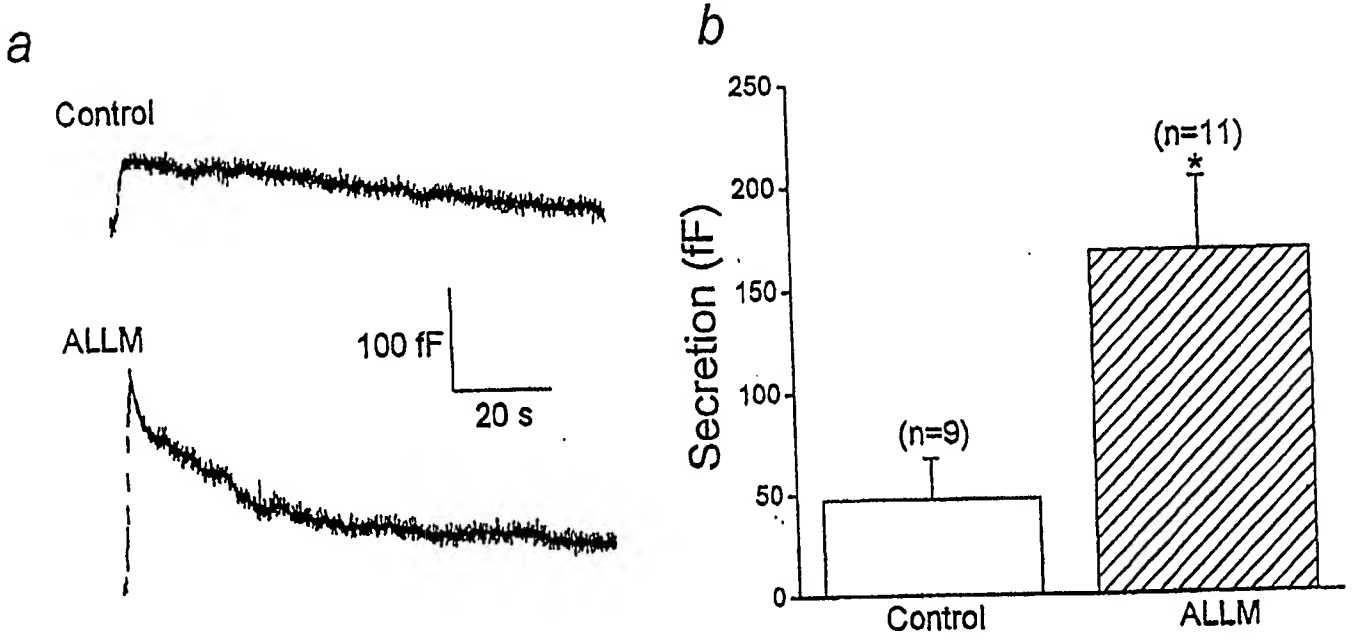

a
Control
ALLM
100 fF
20 s
b
Secretion (fF)
250
200
150
100
50
0
(n=11)
*
(n=9)
Control
ALLM

FIG. 10

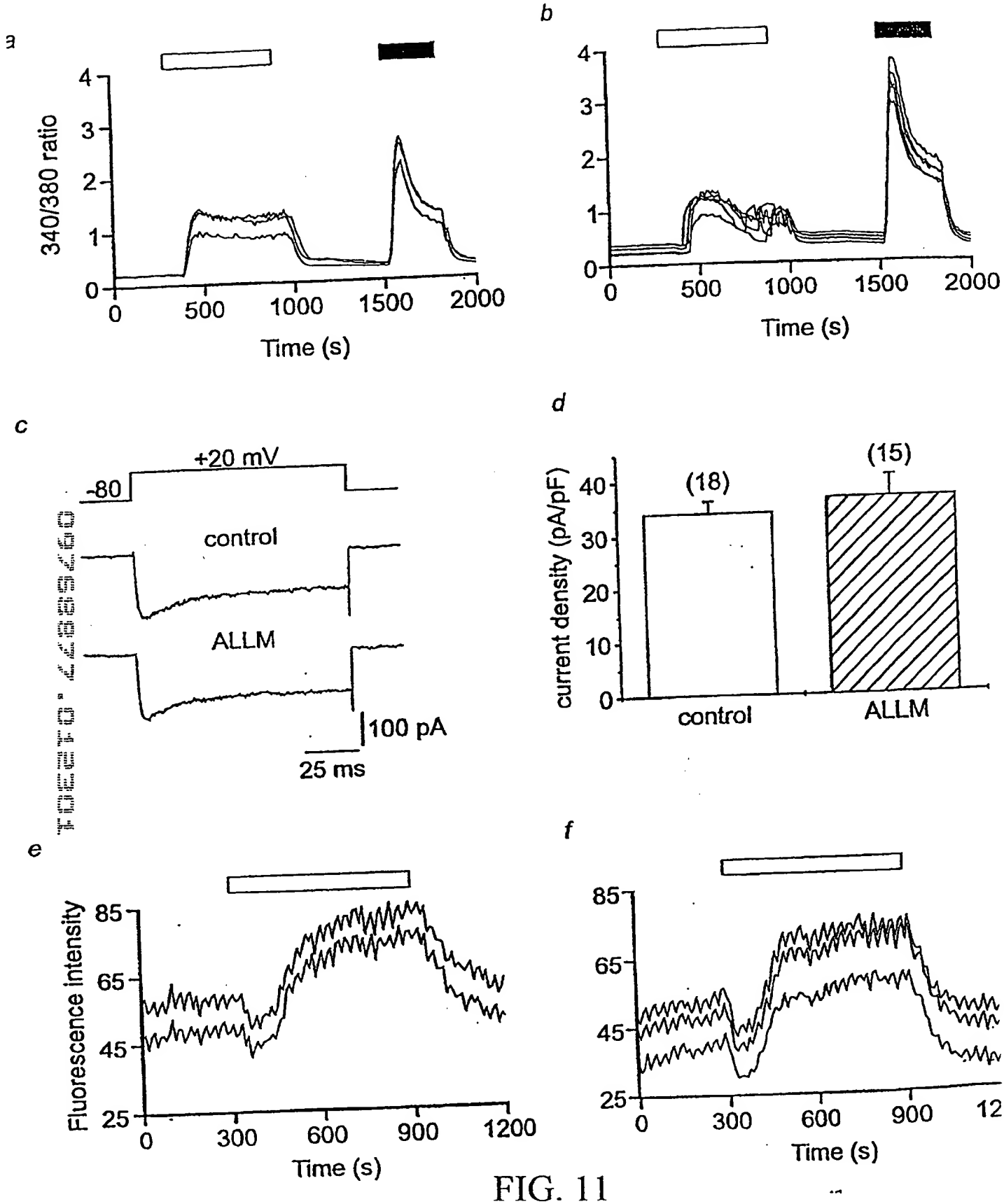

a
340/380 ratio
Time (s)
b
Time (s)
c
+20 mV
-80
control
ALLM
100 pA
25 ms
d
current density (pA/pF)
(18)
(15)
control
ALLM
e
Fluorescence intensity
Time (s)
f
Time (s)

FIG. 11

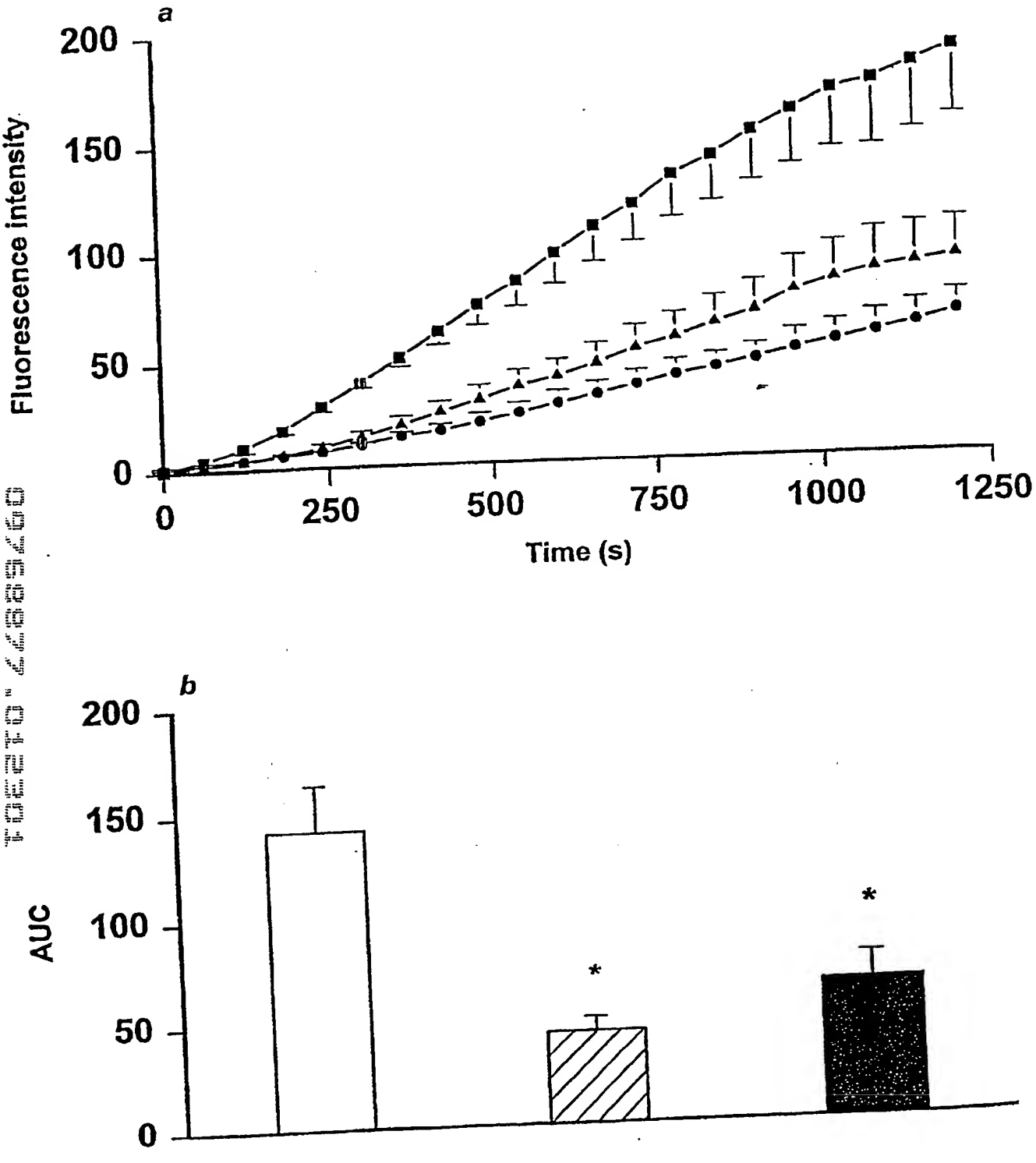

a
200
150
100
50
0
Fluorescence intensity
0
250
500
750
1000
1250
Time (s)
b
200
150
100
50
0
AUC
*
*

FIG. 12

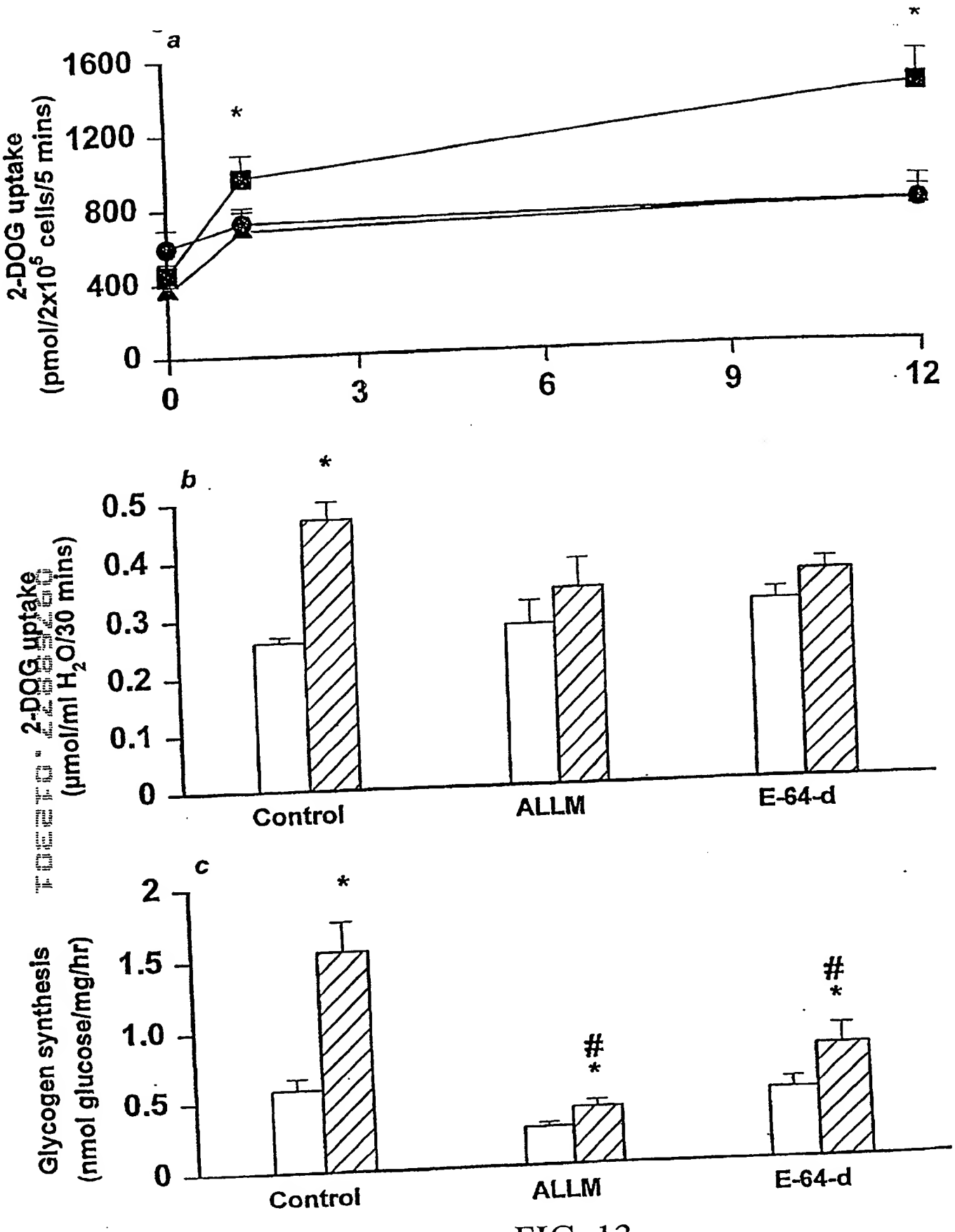

a
2-DOG uptake
(pmol/2x10^5 cells/5 mins)
1600
1200
800
400
0
0
3
6
9
12
*
*
b
2-DOG uptake
(μmol/ml H2O/30 mins)
0.5
0.4
0.3
0.2
0.1
0
*
Control
ALLM
E-64-d
c
Glycogen synthesis
(nmol glucose/mg/hr)
2
1.5
1.0
0.5
0
*
#
*
#
*
Control
ALLM
E-64-d

FIG. 13

Fig14. Effect of 48 hours exposure of islets to calpain inhibitors on insulin secretion

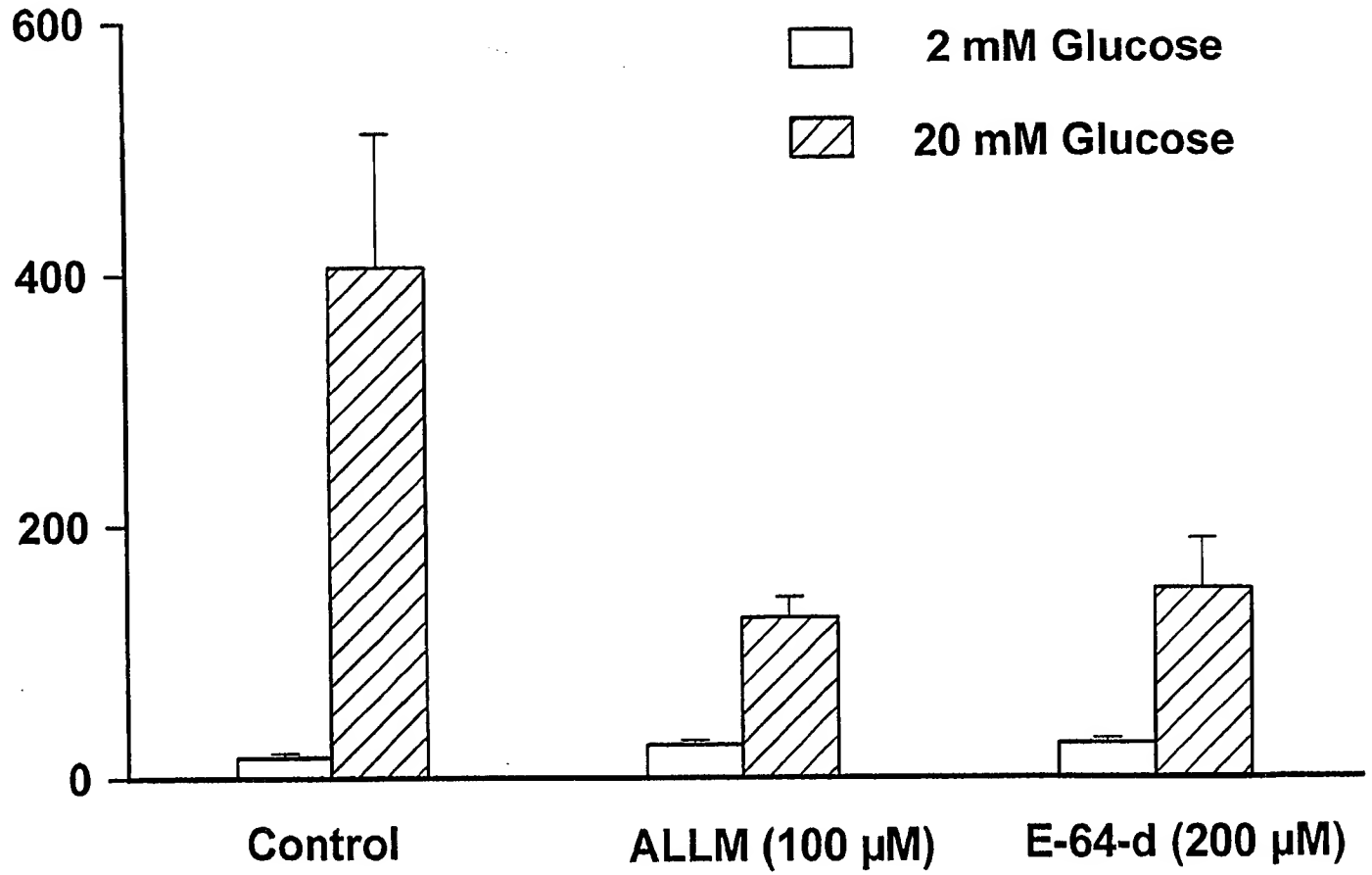


Fig 15 Insulin content in 48 hour cultured islets (n=4)

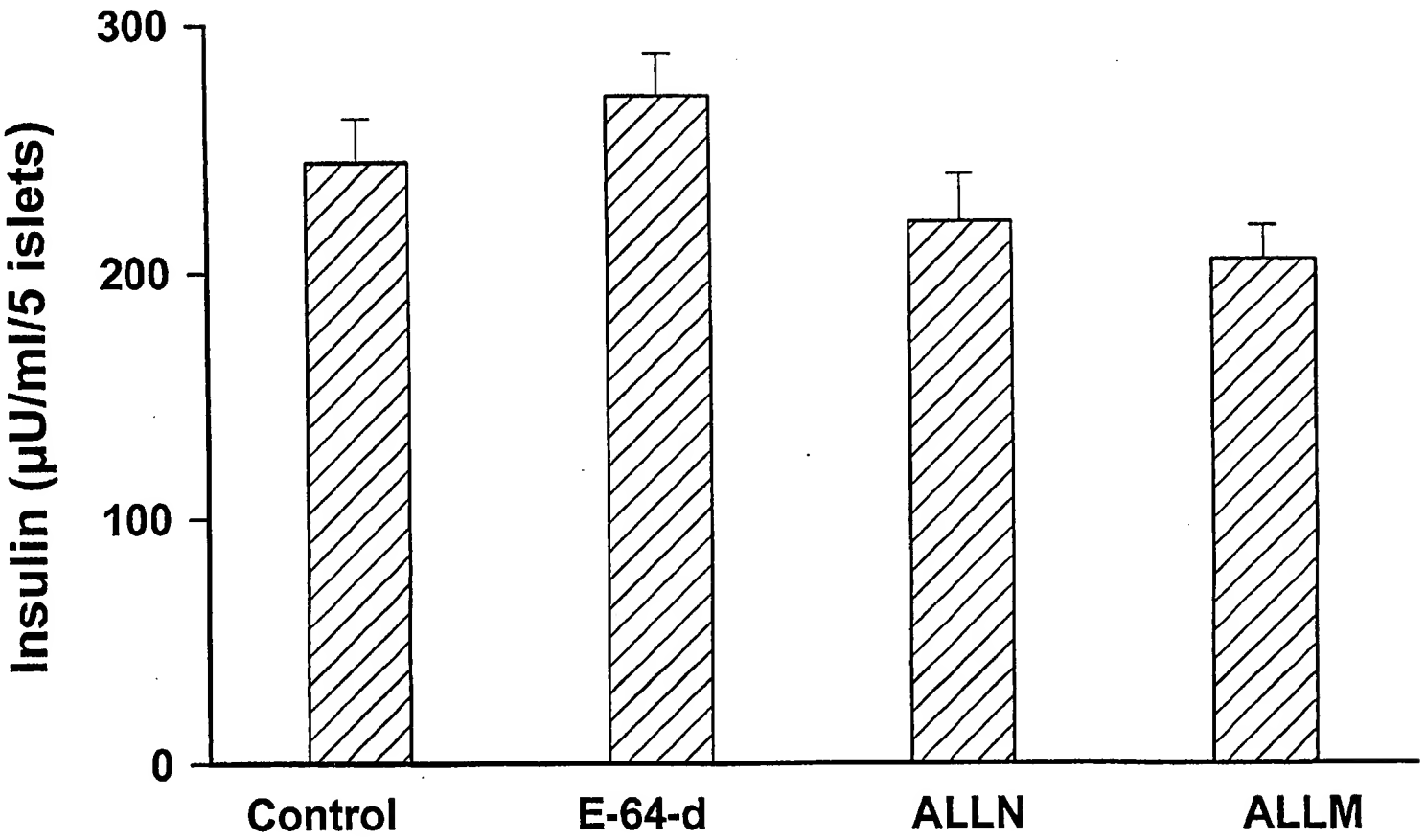


Fig16 ALLM dose response in 48 hour treated islets

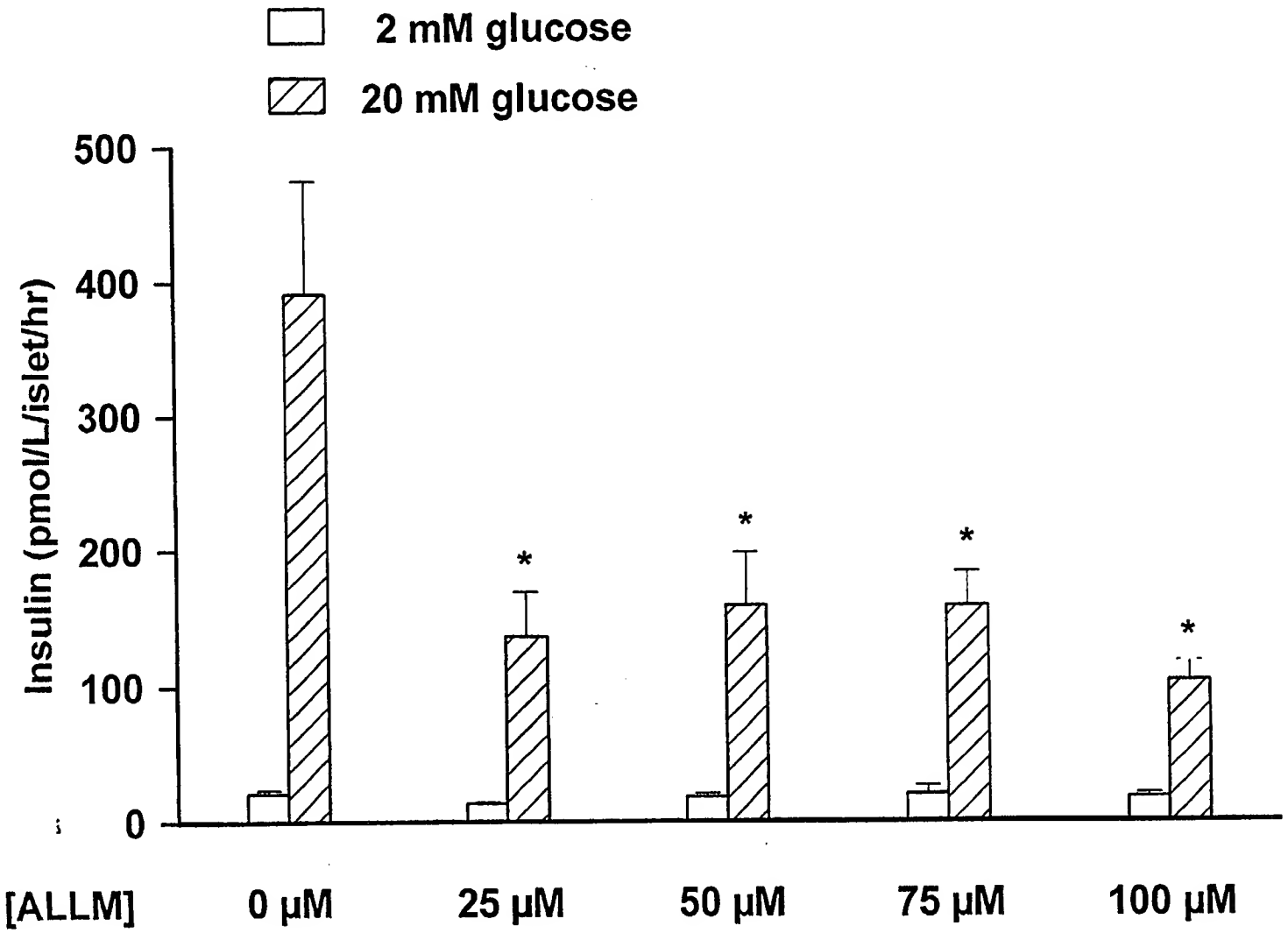
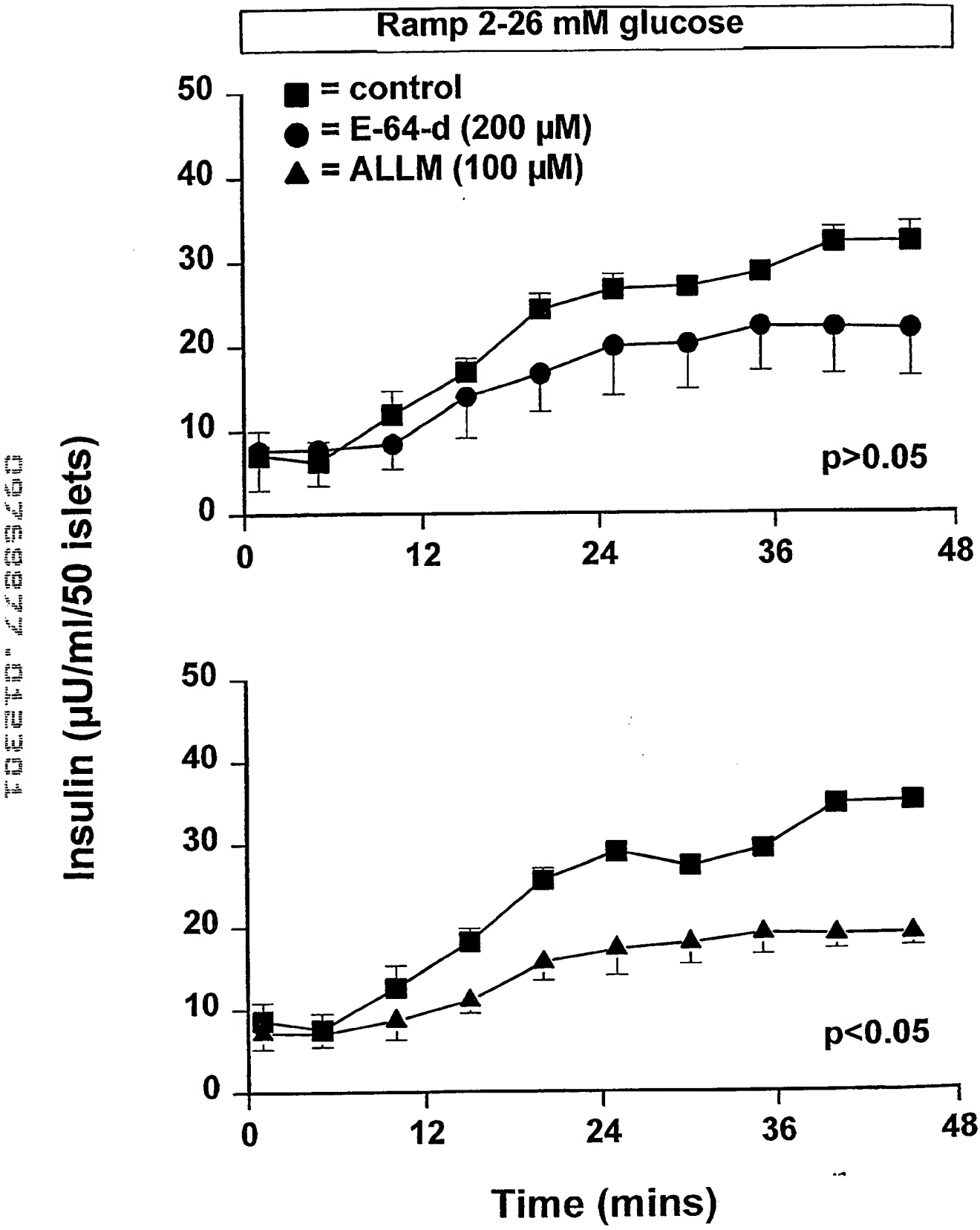


Fig 17. Perifusion of 48 hour cultured islets (n=4)



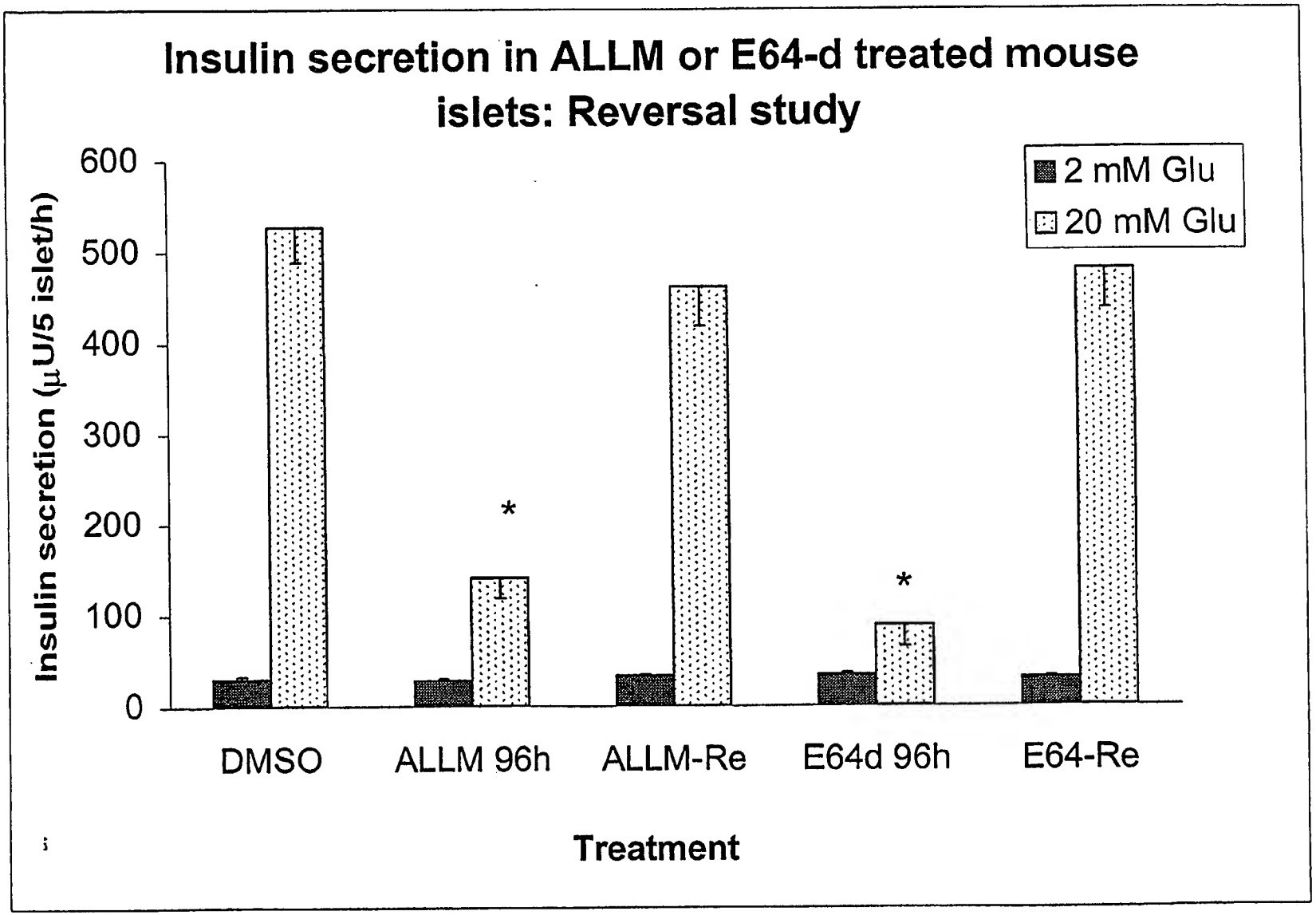
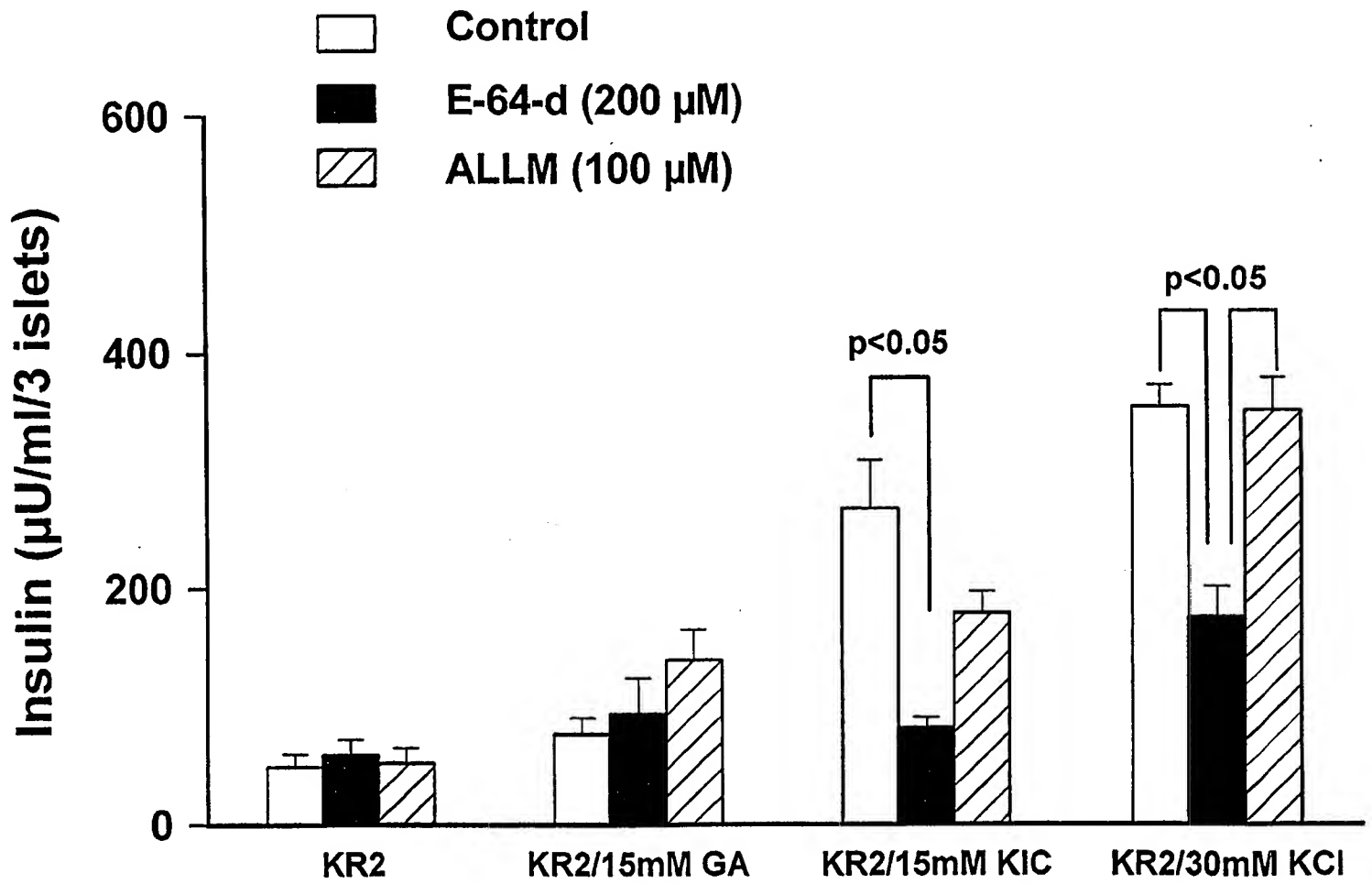

Insulin secretion in ALLM or E64-d treated mouse islets: Reversal study
Insulin secretion (μU/5 islet/h)
600
500
400
300
200
100
0
2 mM Glu
20 mM Glu
*
*
DMSO
ALLM 96h
ALLM-Re
E64d 96h
E64-Re
Treatment

FIG. 18

Fig 19. Insulin secretion by islets following exposure to calpain inhibitors for 48 hrs

(n=6)



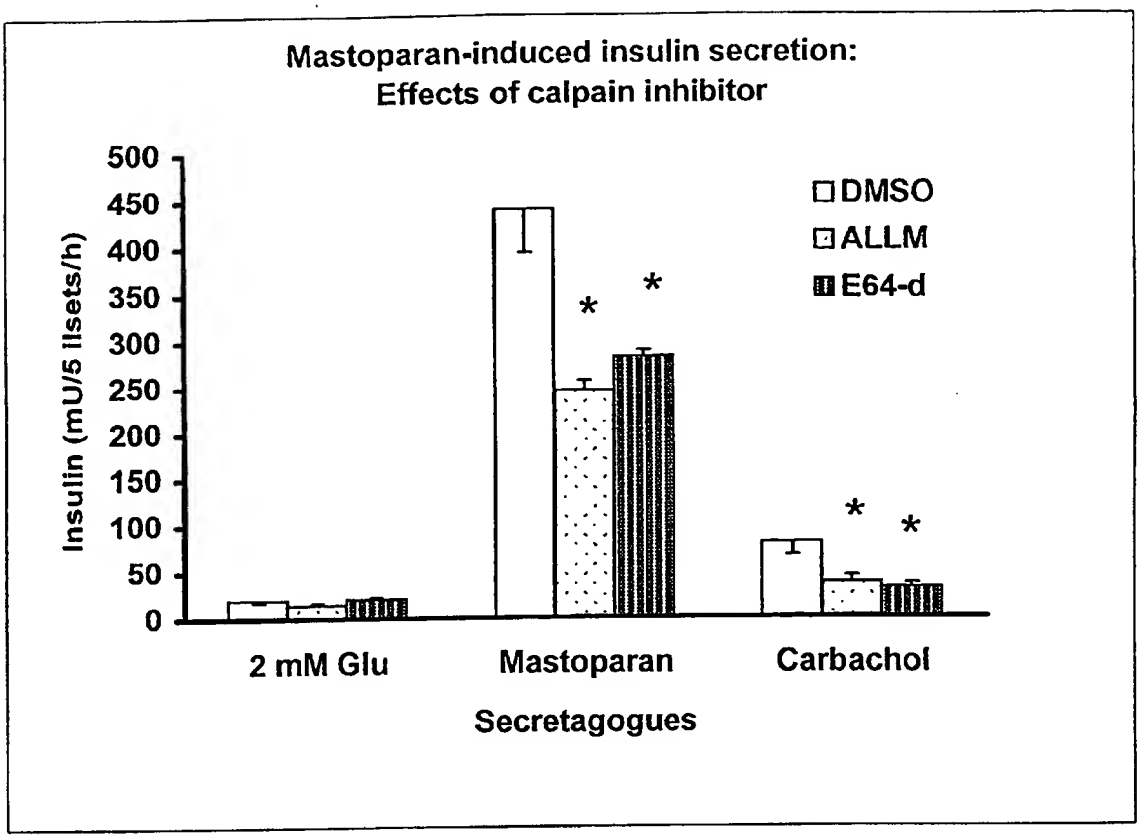
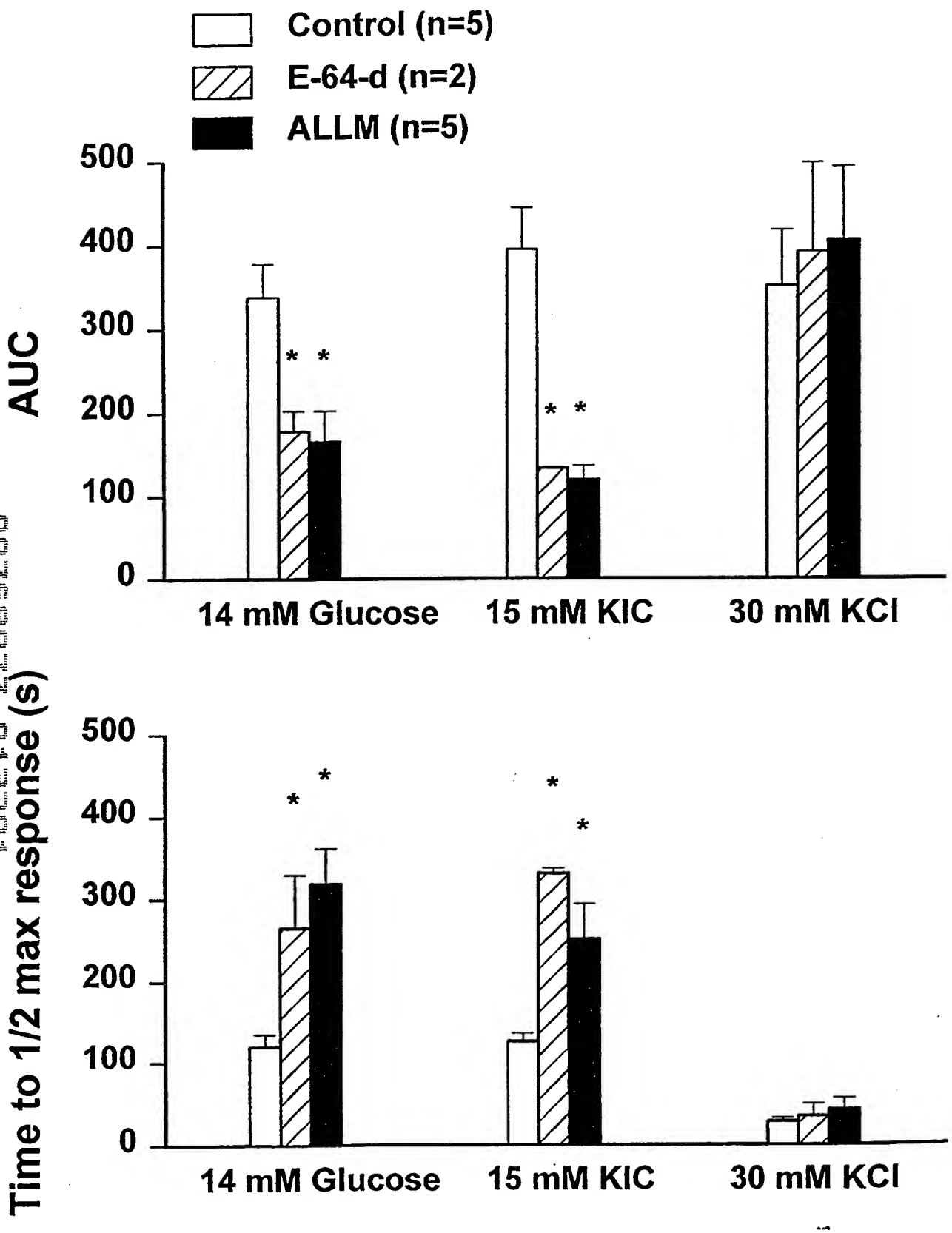

Mastoparan-induced insulin secretion:
Effects of calpain inhibitor
Insulin (mU/5 Ilsets/h)
500
450
400
350
300
250
200
150
100
50
0
DMSO
ALLM
E64-d
*
*
*
*
2 mM Glu
Mastoparan
Carbachol
Secretagogues

FIG. 20

Fig 21. [Ca^{2+}]$_i$ responses to gluc[illegible]e, KIC and KCl



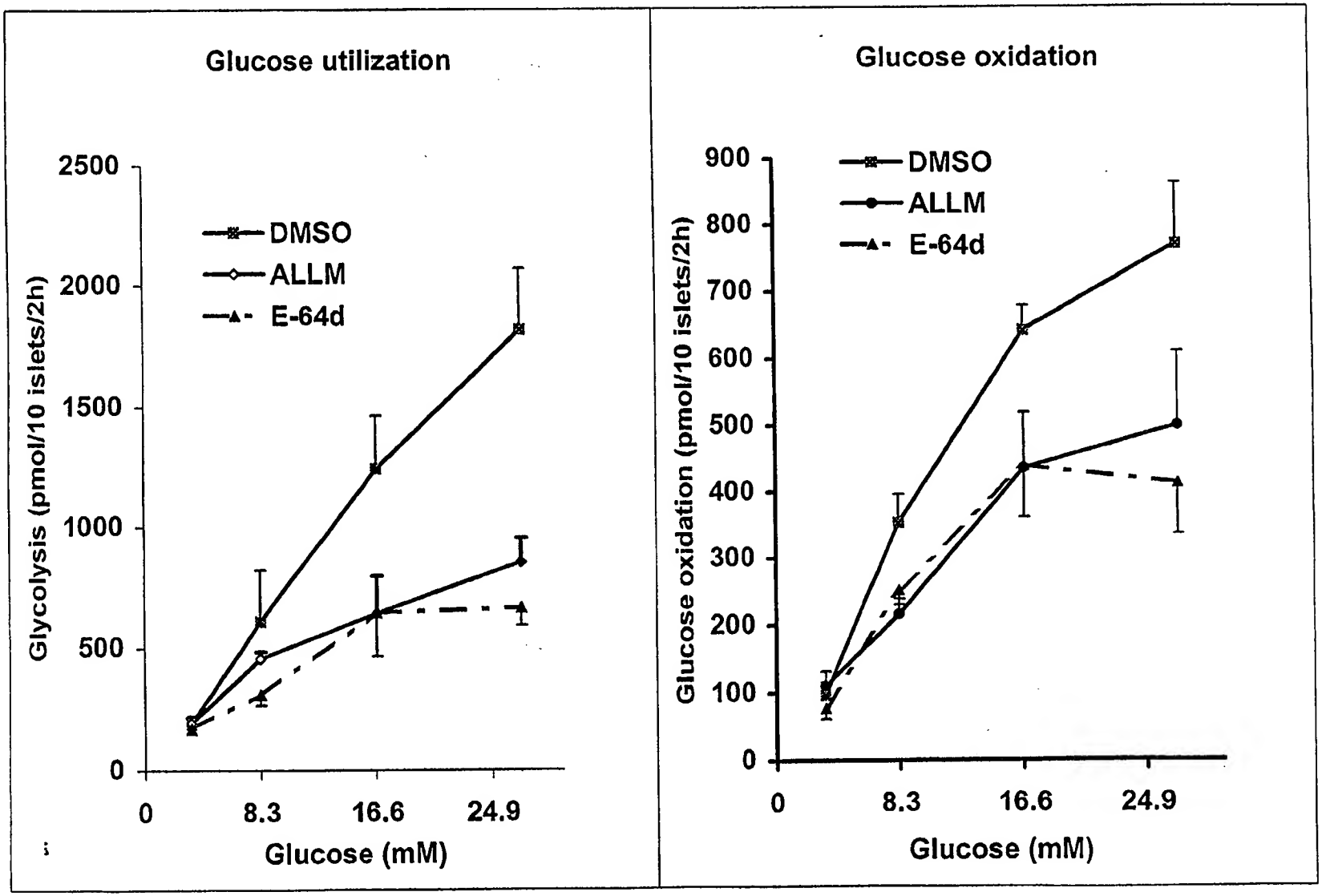
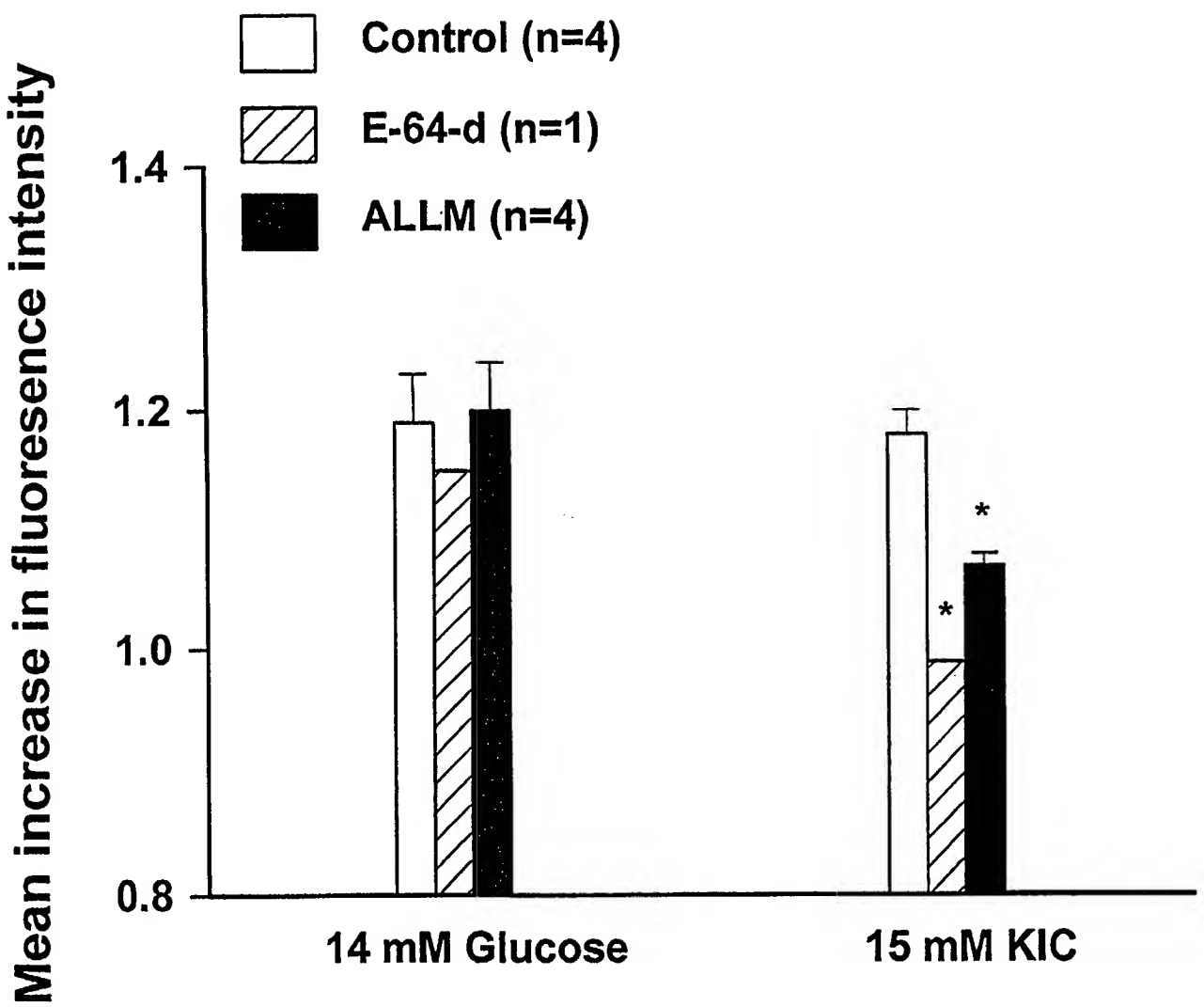

Glucose utilization
Glycolysis (pmol/10 islets/2h)
2500
2000
1500
1000
500
0
DMSO
ALLM
E-64d
0
8.3
16.6
24.9
Glucose (mM)
Glucose oxidation
Glucose oxidation (pmol/10 islets/2h)
900
800
700
600
500
400
300
200
100
0
DMSO
ALLM
E-64d
0
8.3
16.6
24.9
Glucose (mM)

FIG. 22

Fig 23. NAD(P)H responses to glucose and KIC



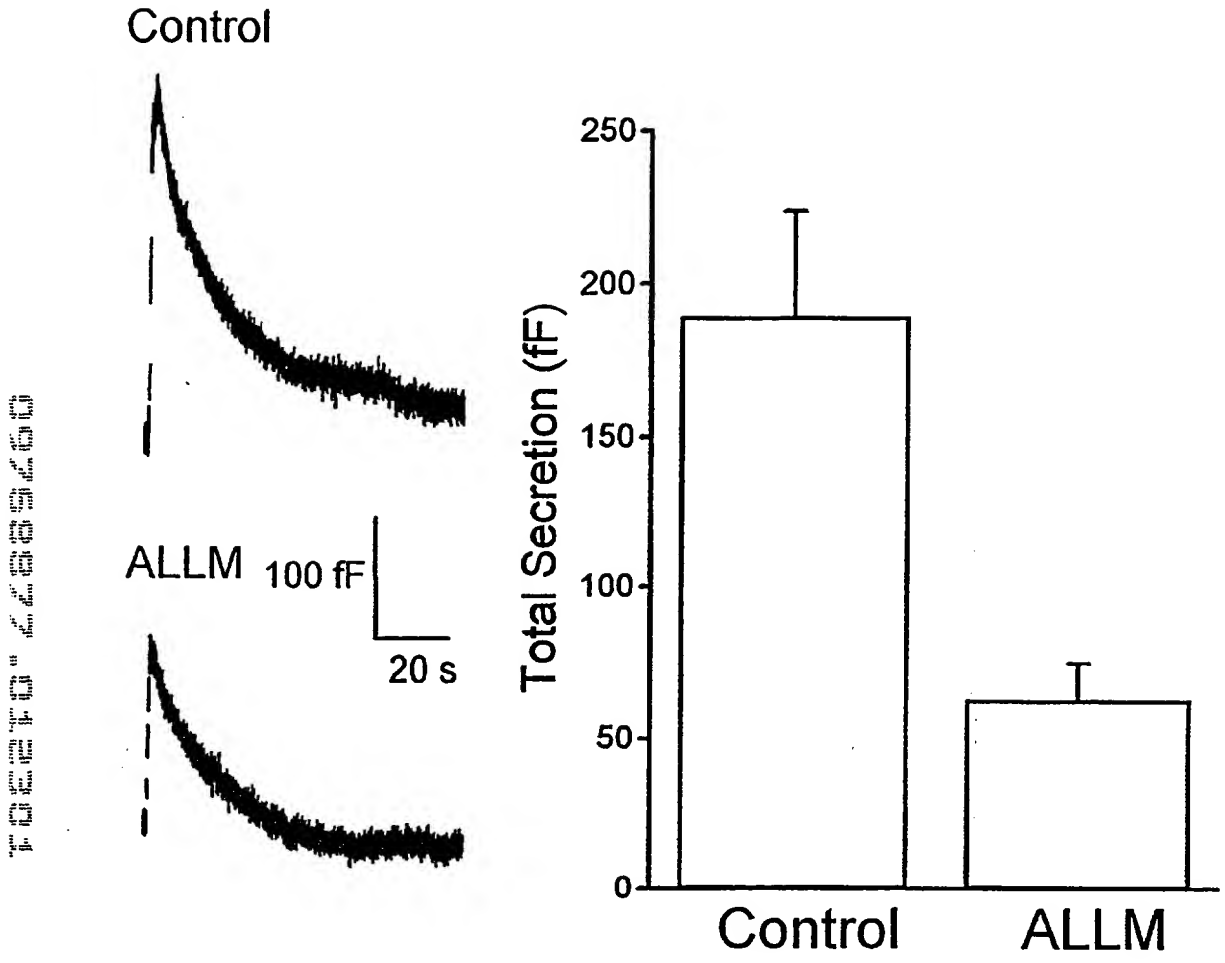
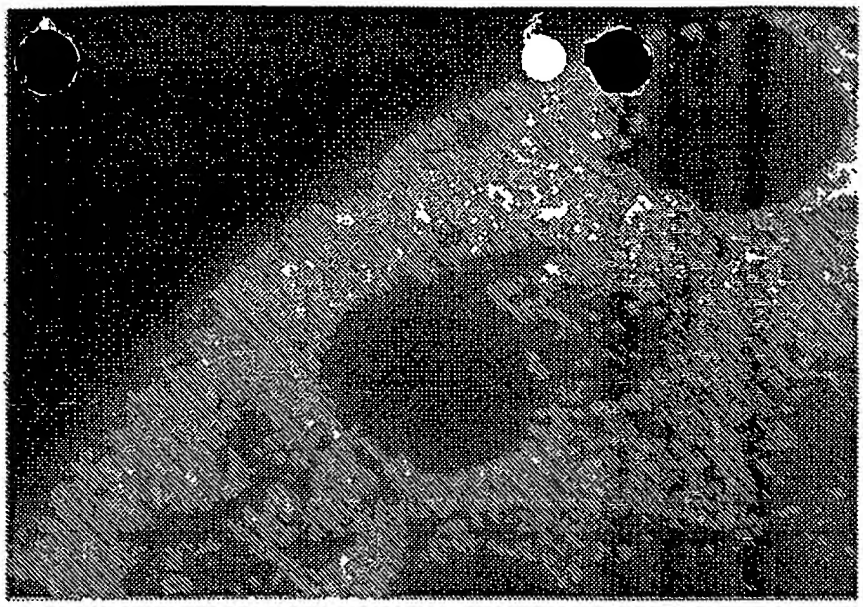
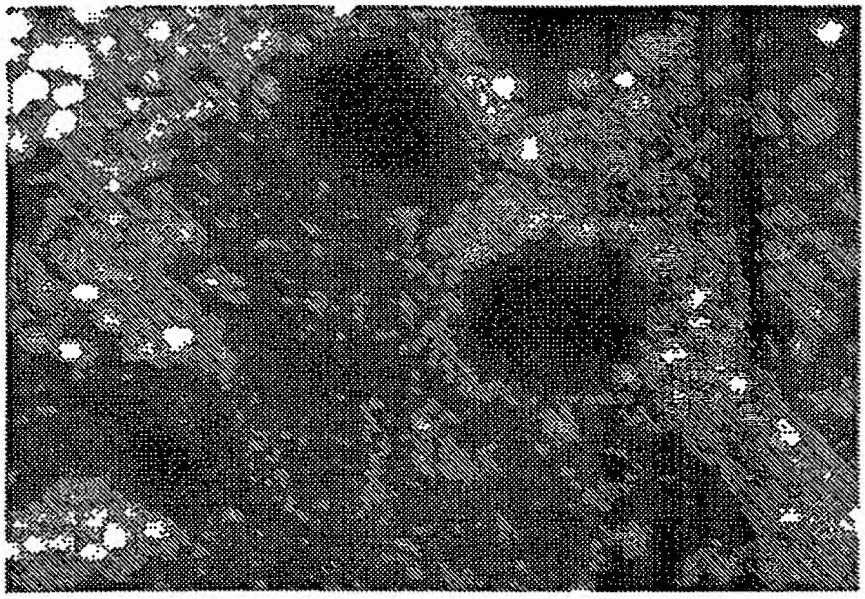


Fig. 24. Measurement of membrane capacitance in isolated β-cells

DMSO



E64d



ALLM

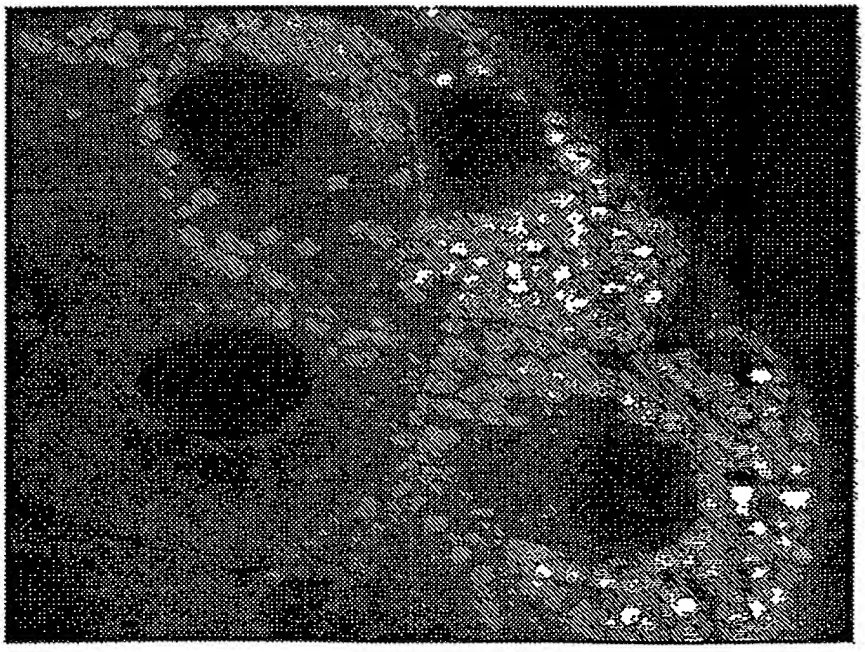


FIG.25

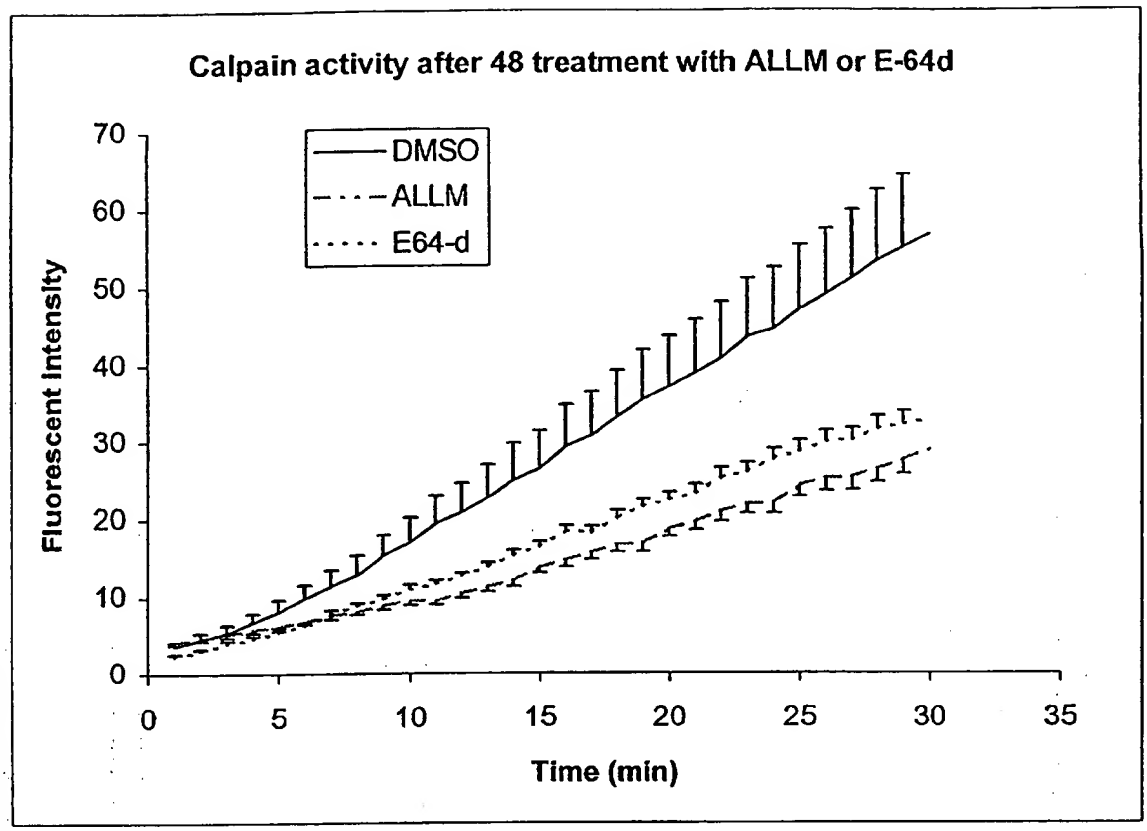

Calpain activity after 48 treatment with ALLM or E-64d
DMSO
ALLM
E64-d
Fluorescent Intensity
Time (min)
0
10
20
30
40
50
60
70
0
5
10
15
20
25
30
35

FIG. 26